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Effects of Dietary Insect Powder Supplementation on Hematological Parameters of Common Carp (*Cyprinus carpio*) Fry

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Abstract: The increasing demand for sustainable protein sources in aquaculture has driven research into insect meal as an alternative to fishmeal for fish nutrition. This study evaluated the effects of dietary insect powder supplementation on blood parameters and liver enzymes in common carp (*Cyprinus carpio*) fry to assess both efficacy and safety of this protein source. Seventy-two common carp fry (average weight 16.65±0.01 g) were randomly distributed into four treatments with six fish per replicate: T0 (control diet), T1 (1% insect powder), T2 (2% insect powder), and T3 (3% insect powder). The insect powder consisted of equal proportions of dried grasshopper and mealworms mixed with a commercial floating diet (30% crude protein, 412 kcal/g gross energy). After 60 days of feeding, blood samples were collected to analyze hematological parameters including red blood cells, hemoglobin, hematocrit, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), platelets, and white blood cells, as well as liver enzymes aspartate aminotransferase (AST), alanine aminotransferase (ALT), and alkaline phosphatase (ALP). All insect powder supplementation treatments significantly improved blood parameters compared to the control, with treatment effectiveness following the order T3 > T2 > T1 > T0. Treatment T3 achieved the highest values for red blood cells, hemoglobin, and hematocrit. Notably, liver enzyme levels showed no significant differences between treatments, indicating the absence of hepatotoxicity. The results demonstrate that insect powder supplementation, particularly at 3% inclusion level, effectively enhances hematological parameters in common carp fry without causing liver damage, supporting its potential as a safe and beneficial alternative protein source in aquaculture feeds.

Keywords: Common carp; insect meal; hematological parameters; alternative protein; aquaculture nutrition

1. Introduction

Aquaculture has emerged as the fastest-growing food production sector globally, contributing significantly to meeting the increasing demand for protein-rich foods [1]. However, the industry faces substantial challenges regarding sustainable feed ingredients, particularly the reliance on fishmeal as a primary protein source [2]. Fishmeal, derived from wild-caught fish, is characterized by its exceptional nutritional profile, featuring a high protein content, a balanced amino acid composition, and excellent palatability [3]. Nevertheless, the ongoing reliance on fishmeal poses significant challenges related to environmental sustainability, resource depletion, and economic feasibility, particularly as global fishmeal production has stagnated while aquaculture production continues to rise [4-5]. The urgent need for alternative protein sources has driven extensive research into various substitutes, including plant-based proteins, single-cell proteins, and, more recently, insect meal [6, 7].

Among these alternatives, insect meal has garnered considerable attention due to its superior nutritional characteristics, including a high protein content (40-70%), a favorable amino acid profile, essential fatty acids, vitamins, and minerals [8-9]. Insects such as black soldier fly larvae, mealworms, crickets, and grasshoppers have shown promising results as replacements for fishmeal in various aquaculture species [10-11]. Additionally, insect production offers environmental advantages, including lower greenhouse gas emissions, reduced land and water requirements, and the ability to utilize organic waste streams [12].

Although many studies have investigated the effects of insect meal on growth performance and feed utilization in fish [13-14], there remains a limited understanding of the physiological responses, specifically hematological parameters and liver function, which serve as crucial indicators of fish health and nutritional status [15]. Blood parameters, including red blood cell count, hemoglobin concentration, and hematocrit, reflect the oxygen-carrying capacity and overall physiological condition of fish [16]. Similarly, liver enzymes such as aspartate aminotransferase (AST), alanine aminotransferase (ALT), and alkaline phosphatase (ALP) serve as sensitive biomarkers for hepatotoxicity and metabolic stress [17-18]. The common carp (Cyprinus carpio) is one of the most important aquaculture species globally, accounting for a significant portion of freshwater fish production [19]. Despite its economic importance, research on the effects of insect meal supplementation on blood parameters and liver function in common carp remains limited. Therefore, the primary objective of this study was to investigate the impact of dietary insect powder (a mixture of grasshopper and mealworm) supplementation at varying inclusion levels on hematological parameters and liver enzymes in common carp fry, thereby providing insights into both the nutritional benefits and safety of insect-based feeds.

2. Materials and Methods

2.1 Experimental Design and Fish Management

2.1.1 Experimental Fish

A total of 72 common carp (*Cyprinus carpio* L.) fry were obtained from a private hatchery in Al-Mahawil District, Babil Governorate, Iraq. The fish had an initial average weight of 16.65 ± 0.01 g. Upon arrival at the experimental facility, fish were gradually thermally acclimated and subsequently treated with a 0.3% saline bath for 5 minutes to eliminate potential pathogens that could interfere with the experimental outcomes. Following treatment, the fish were maintained in storage tanks before being randomly distributed into experimental units.

2.1.2 Experimental Setup

The fish were randomly assigned to four experimental treatments, with three replicates per treatment and six fish per replicate, following a completely randomized design (CRD). The experimental treatments were: T0 (control diet without insect powder supplementation), T1 (basal diet + 1% insect powder), T2 (basal diet + 2% insect powder), and T3 (basal diet + 3% insect powder).

2.2 Rearing System and Environmental Conditions

2.2.1 Facility Description

The growth experiment was conducted at the Animal House Laboratory, Al-Qadisiyah University, College of Education, Department of Biology, from March 17, 2025, to May 15, 2025. The experimental system consisted of 12 glass aquariums with dimensions of 50 cm × 50 cm × 50 cm, providing a volume of 0.125 m^3 and a capacity of 125 liters each. The aquariums were arranged in a U-shaped configuration and covered with plastic mesh to prevent fish from escaping.

2.2.2 Water System and Equipment

Each aquarium was equipped with a 25-watt aeration pump and a 50-watt water heater to maintain optimal environmental conditions. The water supply was provided through 3/4-inch diameter pipes connected to a 1/2 horsepower water pump, which was linked to a 500-liter plastic storage tank. Water was allowed to stand for more than 8 hours before use to facilitate the removal of chlorine. An additional 1,000-liter tank served as a secondary water storage and supply reservoir. Water drainage was accomplished

through hand pumps connected to 1-inch diameter pipes, which led to a main drainage system. The facility was equipped with an electrical inverter to ensure a continuous power supply during potential outages.

2.2.3 Water Quality Monitoring

The following physicochemical parameters were monitored throughout the experimental period: water temperature, dissolved oxygen concentration, pH, salinity, and total hardness. Measurements were conducted using standard protocols to ensure optimal environmental conditions for the growth and development of common carp.

2.3 Experimental Diets and Feed Preparation

2.3.1 Basal Diet

A commercial floating diet (Fardaneh brand, Iranian origin) was used as the basal diet. The diet contained 30% crude protein, 18% fat, 9% crude fiber, 9% ash, 22% nitrogen-free extract, 1.5% phosphorus, and had a gross energy content of 412 kcal/g. Vitamin supplementation included 250 mg vitamin C, 200 mg vitamin E, 2000 I.U. vitamin D3, and 8000 I.U. vitamin A per kilogram of feed.

2.3.2 Insect Powder Preparation

Commercial dried grasshopper and mealworm powder, typically used for feeding ornamental birds and fish, was obtained from local markets. The two insect types were mixed in equal proportions and continuously blended to ensure homogeneity. The mixture was then finely ground, and samples were submitted to laboratories affiliated with the Ministry of Science and Technology for analysis of their chemical composition. The insect powder contained 41.26% crude protein, 12.06% fat, 7.15% crude fiber, 3.65% ash, and 29.14% nitrogen-free extract on a dry matter basis (93.26%).

2.3.3 Experimental Diet Formulation

The basal Fardaneh diet was ground into a fine powder, and 10-kg portions were allocated for each experimental treatment. Insect powder was incorporated according to the experimental design at inclusion levels of 0%, 1%, 2%, and 3% for treatments T0, T1, T2, and T3, respectively. Each mixture was thoroughly blended to ensure homogeneous distribution of the insect powder. Water was added at a rate of 350-400 ml per kg until a solid paste consistency was achieved. The mixture was then processed through a National-type grinding machine with 2-3 mm holes to form feed pellets. The pellets were air-dried and exposed to sunlight until completely dry, then stored in sealed bags until use.

Table 1. The chemical composition of the Fardaneh diet

Elements	Unit
Dry matter (%)	88
Crude protein (%)	30
Fat (%)	18
Crude fiber (%)	9
Ash (%)	9
Nitrogen-free extract (%)	22
Phosphor (%)	1.5
Vit. C (mg)	250
Vit. E (mg)	200
Vit. D3 (I.U.)	2000
Vit. A (I.U.)	8000
Gross energy (Kcal/g)*	412

*Total energy = (% protein x 5.4) + (% fat x 9) + (% nitrogen-free extract x 4)

2.4 Sample Collection and Analysis

2.4.1 Blood Sampling

After 60 days of experimental feeding, blood samples were collected from two fish per treatment. Blood was drawn from the caudal vein using 3 ml plastic syringes, with sample volumes ranging from 0.5 to 1.0 ml per fish. For hematological analysis, blood samples were immediately transferred to tubes containing ethylenediaminetetraacetic acid (EDTA) as an anticoagulant. For serum biochemical analysis, separate blood samples were collected in vacuum gel tubes without anticoagulants to facilitate the separation of serum.

Table 2. Powder of insect mixture

Element	Percentage (%)
Dry matter	93.26
Crude protein (N*6.25)	41.26
Fat	12.06
Crude fiber	7.15
Ash	3.65
Nitrogen-free extract	29.14
Total	100%

2.4.2 Hematological and Biochemical Analysis

Hematological parameters analyzed included red blood cell count, white blood cell count, hemoglobin concentration, hematocrit, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), and platelet count. Serum biochemical analysis focused on liver enzyme activities, specifically aspartate aminotransferase (AST), alanine aminotransferase (ALT), and alkaline phosphatase (ALP). All analyses were performed at the Zoo Center using a Seamaty Hematology Analyzer (SMT 120 VP, China).

2.5 Statistical Analysis

Data were analyzed using SPSS software version 26 following a completely randomized design (CRD). Significant differences between treatment means were determined using Duncan's multiple range test [20] at a significance level of $p \leq 0.05$. Results are presented as means ± standard error.

3. Results and Discussion

3.1 Environmental Parameters

Throughout the 60-day experimental period, all monitored water quality parameters remained within optimal ranges for common carp cultivation. Water temperature ranged between 20-21°C, dissolved oxygen levels were maintained at 7.1-7.4 mg/L, pH values recorded between 7.1-7.23, salinity ranged from 1.27-1.8 g/L, and total hardness values were between 450-455.2 mg/L. These environmental conditions are consistent with established requirements for common carp aquaculture [21, 22], ensuring that any observed differences in physiological parameters could be attributed to dietary treatments rather than environmental stress factors. According to established guidelines, common carp can tolerate temperature ranges of 3-35°C [23], minimum dissolved oxygen levels of 3 mg/L [24], salinity levels up to 14 g/L [25], pH ranges of 6.5-9.5 [26], and total hardness up to 620 mg $CaCO_3$/L [27]. The recorded parameters in this study were well within these acceptable ranges, confirming optimal rearing conditions throughout the experimental period.

3.2 Hematological Parameters

3.2.1 Red Blood Cell Parameters

The incorporation of insect powder in common carp diets resulted in significant improvements in red blood cell-related parameters (Table 3). Treatment T3 (3% insect powder) demonstrated the highest red blood cell count ($1.94 \pm 0.05 \times 10^6$ cells/mL), followed by T2 ($1.24 \pm 0.01 \times 10^6$ cells/mL), T1 ($0.95 \pm 0.05 \times 10^6$ cells/mL), and T0 ($0.78 \pm 0.02 \times 10^6$ cells/mL), with all supplemented treatments showing significant differences ($p \leq 0.05$) compared to the control. Similarly, hemoglobin concentrations followed the same pattern, with T3 achieving the highest value (11.05 ± 0.05 g/dL), significantly exceeding T2 (10.50 ± 0.29 g/dL), T1 (9.70 ± 0.20 g/dL), and

T0 (7.85 ± 0.55 g/dL). Hematocrit values also demonstrated dose-dependent improvements, with T3 recording 24.91 ± 0.41%, compared to 22.83 ± 0.17%, 21.75 ± 0.25%, and 19.94 ± 0.05% for T2, T1, and T0, respectively. The enhancement in erythropoietic parameters can be attributed to the rich nutritional profile of the insect powder mixture. Grasshoppers and mealworms are excellent sources of vitamin B_{12} [28], which plays a crucial role in erythropoiesis and red blood cell maturation [29]. Additionally, these insects contain substantial amounts of iron and B-complex vitamins [30, 31], which are essential cofactors in the synthesis of hemoglobin and the formation of red blood cells [32].

3.2.2. Red Blood Cell Indices

Mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC) showed similar dose-dependent improvements across treatments. Treatment T3 recorded the highest MCV (161.50 ± 0.50 μL), MCH (32.12 ± 0.09 pg/mL), and MCHC (33.59 ± 0.28%), all of which were significantly higher than those in the control treatment. These improvements in red blood cell indices suggest enhanced erythropoietic activity and the formation of larger, more hemoglobin-rich erythrocytes [33]. The superior amino acid profile of insect protein, particularly the presence of essential amino acids such as leucine, valine, histidine, methionine, lysine, and tyrosine [30, 34], likely contributed to improved hemoglobin synthesis and red blood cell quality. Recent research has demonstrated that amino acid supplementation, particularly leucine, valine, and histidine, significantly influences red blood cell production, while methionine, lysine, and tyrosine contribute directly to hemoglobin synthesis [35].

3.2.3. White Blood Cell and Platelet Counts

White blood cell counts showed significant improvements in treatments T2 (94.96 ± 0.54 × 10^3/mL) and T3 (96.40 ± 0.40 × 10^3/mL) compared to T0 (89.58 ± 0.51 × 10^3/mL) and T1 (91.61 ± 0.61 × 10^3/mL), although no significant difference was observed between T2 and T3. Platelet counts demonstrated a clear dose-response relationship, with T3 achieving the highest count (99.55 ± 0.44 × 10^3/μL), followed by T2, T1, and T0. The enhancement in white blood cell counts suggests improved immune function, likely attributed to the presence of antimicrobial peptides (AMPs) in insect meal [36]. These bioactive compounds, along with chitin derivatives, have been shown to modulate immune responses in aquatic organisms [37]. Furthermore, insect meals contain lauric acid, a medium-chain fatty acid with demonstrated antimicrobial and immunostimulatory properties [38, 39]. The improved platelet counts indicate enhanced hemostatic function and potentially improved stress resistance in fish fed insect-supplemented diets.

Table 3. Some of the studied blood parameters (mean ± standard error) of common carp fry fed on a mixture of insect powder during the duration of the experiment.

Studied parameters	Experimental treatments				Significant level
	T0	T1	T2	T3	
R.B.C (10^6cell/ml)	0.78 ± 0.02^{d}	0.95 ± 0.05^{c}	1.24 ± 0.01^{b}	1.94 ± 0.05^{a}	0.05
Hemoglobin (g/dL)	7.85 ± 0.55^{c}	9.70 ± 0.20^{b}	10.50 ± 0.29^{ab}	11.05 ± 0.05^{a}	0.05
Hematocrit (%)	19.94 ± 0.05^{d}	21.75 ± 0.25^{c}	22.83 ± 0.17^{b}	24.91 ± 0.41^{a}	0.05
M.C.V (μL)	159.50 ± 0.50^{b}	160.30 ± 0.10^{ab}	160.40 ± 0.09^{ab}	161.50 ± 0.50^{a}	0.05
M.C.H (pg/mL)	29.05 ± 0.05^{c}	29.71 ± 0.38^{bc}	31.15 ± 0.85^{ab}	32.12 ± 0.09^{a}	0.05
M.C.H.C (%)	30.59 ± 0.49^{b}	31.65 ± 0.65^{b}	33.80 ± 0.20^{a}	33.59 ± 0.28^{a}	0.05
Platelets (10^3/ μL)	88.50 ± 0.50^{d}	90.81 ± 0.21^{c}	95.51 ± 0.51^{b}	99.55 ± 0.44^{a}	0.05
W.B.C (10^3/ml)	89.58 ± 0.51^{b}	91.61 ± 0.61^{b}	94.96 ± 0.54^{a}	96.40 ± 0.40^{a}	0.05

3.3 Liver Enzyme Activities

3.3.1 Hepatic Safety Assessment

Liver enzyme analysis revealed no significant differences ($p > 0.05$) between treatments for aspartate aminotransferase (AST), alanine aminotransferase (ALT), and alkaline phosphatase (ALP) activities (Table 4). AST values ranged from 67.47 ± 0.47 to 68.75 ± 0.25 I.U./L, ALT values from 13.00 ± 0.99 to 14.65 ± 0.45 I.U./L, and ALP values from 25.60 ± 0.60 to 27.50 ± 0.50 I.U./L across all treatments. These results are particularly

significant as they demonstrate the hepatic safety of insect powder supplementation at the tested inclusion levels. Liver enzymes, particularly AST and ALT, serve as sensitive biomarkers for hepatocellular damage and metabolic stress [40, 41]. The absence of elevated enzyme activities indicates that insect powder supplementation did not induce hepatotoxicity or liver dysfunction, even at the highest inclusion level of 3%.

3.3.2. Metabolic Implications

The maintained liver enzyme activities within normal physiological ranges suggest that insect protein was efficiently utilized without causing metabolic stress. This finding is consistent with previous studies demonstrating the safety of moderate insect meal inclusion in fish diets [42, 43]. The absence of hepatotoxic effects may be attributed to the high digestibility and bioavailability of insect proteins, which reduces the metabolic burden on the liver compared to some plant-based protein sources that may contain anti-nutritional factors.

Table 4. Studied Liver enzymes (mean ± standard error) of common carp fry fed on a mixture of insect powder during the duration of the experiment.

Studied parameters	Experimental treatments				Significant level
	T0	T1	T2	T3	
A.S.T (I.U./L)	67.75 ± 0.75	68.50 ± 0.50	67.47 ± 0.47	68.75 ± 0.25	NS
A.L.T (I.U./L)	13.75 ± 0.75	13.00 ± 0.99	13.65 ± 0.65	14.65 ± 0.45	NS
A.L.P (I.U./L)	25.60 ± 0.60	26.50 ± 0.49	27.50 ± 0.50	27.25 ± 0.25	NS

3.4 Dose-Response Relationships and Optimal Inclusion Levels

The study demonstrated clear dose-dependent responses across most measured parameters, with treatment effectiveness following the order T3 > T2 > T1 > T0. This pattern suggests that within the tested range (0-3%), higher inclusion levels of insect powder provided greater physiological benefits without compromising fish health or liver function. The superior performance of the 3% inclusion level may be attributed to the cumulative effects of enhanced protein quality, improved micronutrient availability, and the presence of bioactive compounds. However, future studies should investigate higher inclusion levels to determine the optimal supplementation rate and identify potential threshold effects beyond which benefits may plateau or adverse effects may occur.

3.5 Mechanistic Considerations and Future Research Directions

The observed improvements in hematological parameters likely result from multiple synergistic mechanisms. The high-quality protein and essential amino acids in insect meal support enhanced protein synthesis for hemoglobin and cellular components. The presence of bioactive compounds, such as antimicrobial peptides and chitin derivatives, may contribute to improved gut health and enhanced nutrient absorption, thereby indirectly supporting a better physiological status [44-45]. Future research should focus on investigating the underlying molecular mechanisms responsible for these improvements, including gene expression studies related to erythropoiesis and immune function. Additionally, longer-term studies are needed to evaluate the sustained effects of insect meal supplementation and to determine optimal feeding strategies for different growth stages of common carp. The environmental sustainability implications of insect meal utilization in aquaculture also warrant further investigation, particularly regarding life cycle assessments and economic feasibility compared to conventional protein sources. Integration of insect farming with aquaculture operations could potentially create circular production systems that enhance overall sustainability and resource efficiency.

4. Conclusions

This study investigated the hypothesis that dietary insect powder supplementation would enhance hematological parameters in common carp fry without causing hepatotoxicity. The results conclusively demonstrate that incorporating a mixture of grasshopper and mealworm powder at inclusion levels of 1%, 2%, and 3% significantly improved blood parameters in a dose-dependent manner, with the 3% inclusion level yielding optimal results. The most significant findings include substantial improvements in red blood cell count, hemoglobin concentration, hematocrit, and red blood cell indices (MCV, MCH, MCHC) across all

supplemented treatments compared to the control. Additionally, white blood cell and platelet counts were enhanced, suggesting improved immune function and hemostatic capacity. Critically, liver enzyme activities (AST, ALT, ALP) remained within normal physiological ranges across all treatments, confirming the hepatic safety of insect powder supplementation at the tested inclusion levels. This research contributes valuable evidence to the growing body of knowledge supporting insect meal as a safe and effective alternative protein source in aquaculture. The findings demonstrate that insect powder not only serves as a nutritionally adequate fishmeal substitute but also actively enhances physiological performance indicators in common carp fry. The absence of hepatotoxic effects addresses significant safety concerns regarding novel feed ingredients in aquaculture. Several limitations should be acknowledged, including the relatively short experimental duration (60 days), the limited range of inclusion levels tested (0-3%), and the focus on a single fish species and life stage. Additionally, the study did not investigate the underlying molecular mechanisms responsible for the observed physiological improvements. Future research directions should include longer-term feeding trials to assess sustained effects and potential cumulative benefits, investigation of higher inclusion levels to determine optimal supplementation rates, evaluation across different fish species and life stages, and molecular-level studies to elucidate the mechanisms underlying enhanced hematological performance. Economic feasibility analyses and life cycle assessments would further support the practical implementation of insect meal in commercial aquaculture operations. Overall, this study provides compelling evidence that insect powder supplementation represents a promising strategy for enhancing fish health while supporting sustainable aquaculture development.

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